

AD \_\_\_\_\_

Award Number: DAMD17-99-1-9125

TITLE: Functional Imaging of Multidrug Resistance in Breast  
Cancer with PET

PRINCIPAL INVESTIGATOR: Alan Packard, Ph.D.

CONTRACTING ORGANIZATION: Children's Hospital Corporation  
Boston, Massachusetts 02115

REPORT DATE: August 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

DTIC QUALITY INSPECTED 4

20010108 155

**REPORT DOCUMENTATION PAGE**Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

**1. AGENCY USE ONLY (Leave blank)****2. REPORT DATE**

August 2000

**3. REPORT TYPE AND DATES COVERED**

Annual (1 Aug 99 - 31 Jul 00)

**4. TITLE AND SUBTITLE**

Functional Imaging of Multidrug Resistance in Breast Cancer with PET

**5. FUNDING NUMBERS**

DAMD17-99-1-9125

**6. AUTHOR(S)**

Alan Packard, Ph.D.

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**Children's Hospital Corporation  
Boston, Massachusetts 02115**8. PERFORMING ORGANIZATION  
REPORT NUMBER****E-MAIL:**

alan.packard@tch.harvard.edu

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012**10. SPONSORING / MONITORING  
AGENCY REPORT NUMBER****11. SUPPLEMENTARY NOTES****12a. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for public release; distribution unlimited

**12b. DISTRIBUTION CODE****13. ABSTRACT (Maximum 200 Words)**

The objective of this project is the development of a PET radiopharmaceutical for the evaluation of multidrug resistance (mdr) in breast cancer. Multidrug resistance is resistance of a lesion to a specific class of drugs that includes many of the chemotherapeutics that are most effective against breast cancer and is characterized by the increased concentration of P-glycoprotein (Pgp), a 170 kD transmembrane glycoprotein, which reduces the intracellular concentration of these drugs to non-toxic levels. Lipophilic cationic complexes such as  $^{99m}\text{Tc}$ -MIBI are substrates for Pgp and are now being studied for evaluation of mdr function *in vivo*. We are currently carrying out *in vivo* and *in vitro* studies of lipophilic cationic  $^{64}\text{Cu}$ -based PET radiopharmaceuticals as possible PET mdr radiopharmaceuticals. A PET mdr tracer will provide significant advantages over  $^{99m}\text{Tc}$ -MIBI, and the half-life of  $^{64}\text{Cu}$  (12.7 h) is better matched to the apparent biological half-life of the mdr process (~240 min.) than are other PET radionuclides (e.g.,  $^{11}\text{C}$ ,  $T_{1/2} = 11$  min). These radiopharmaceuticals may provide information about the mdr status of breast cancer lesions that will allow optimization of treatment protocols, monitoring of the development of acquired resistance, and real-time evaluation of the effectiveness of drugs developed to modulate mdr.

**14. SUBJECT TERMS**Breast Cancer, PET,  $^{64}\text{Cu}$ , Multidrug Resistance**15. NUMBER OF PAGES**

10

**16. PRICE CODE****17. SECURITY CLASSIFICATION  
OF REPORT**

Unclassified

**18. SECURITY CLASSIFICATION  
OF THIS PAGE**

Unclassified

**19. SECURITY CLASSIFICATION  
OF ABSTRACT**

Unclassified

**20. LIMITATION OF ABSTRACT**

Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

## Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusions.....	7
References.....	7
Appendices.....	8

## INTRODUCTION

The objective of this project is the development of a PET (Positron Emission Tomography) radiopharmaceutical for the quantitative functional evaluation of multidrug resistance (mdr) in breast cancer. Multidrug resistance (mdr) is defined as intrinsic or acquired resistance to a specific class of chemotherapeutic drugs that includes many of the most effective chemotherapeutic agents against breast cancer. Multidrug resistance is characterized by overexpression of the *MDR1* gene and increased concentration of its product, P-glycoprotein (Pgp), a 170 kD transmembrane glycoprotein, which acts as an efflux pump to reduce the intracellular concentration of these drugs to non-toxic levels. Lipophilic cationic complexes such as  $^{99m}\text{Tc}$ -MIBI are substrates for Pgp and are now being studied for clinical evaluation of mdr. We are currently carrying out *in vivo* and *in vitro* studies of lipophilic cationic  $^{64}\text{Cu}$ -based PET radiopharmaceuticals as possible PET mdr radiopharmaceuticals. A PET mdr tracer will provide significant advantages over  $^{99m}\text{Tc}$ -MIBI (e.g., straightforward attenuation corrections, higher spatial resolution, greater sensitivity, and the ability to perform quantitative studies), and the half-life of  $^{64}\text{Cu}$  (12.7 h) is better matched to the apparent biological half-life of the mdr process (~240 min.) than are other PET radionuclides such as  $^{11}\text{C}$  ( $T_{1/2}$  = 11 min). Furthermore,  $^{11}\text{C}$ -based radiopharmaceuticals are only available at a limited number of institutions and, because of the short half-life of  $^{11}\text{C}$ , can not be shipped to other institutions. These radiopharmaceuticals will provide real-time information about the mdr status of breast cancer lesions that may allow optimization of treatment protocols, monitoring of the development of acquired resistance, and evaluation of the effectiveness of drugs developed to modulate mdr.

## BODY

The research accomplishments are discussed in terms of each Task outlined in the Revised Statement of Work (6/1/99) relevant to the first 12 months of the Project (8/1/99-7/31/00).

### Task 1. Recruitment and training of research technician, Months 1-3

Ms. Erica A. Guice was hired as a research technician October, 1999, after review of approximately 60 applications and interviews with five finalists. Ms. Guice received her B.S. degree in Cell & Molecular Biology from the University of Michigan in May of 1999 during which time she was a research technician in the Department of Neurology. A copy of her CV is appended to this report.

During the first two to three months of Ms. Guice's appointment she underwent required new employee training sessions including Lab Safety Training, Radiation Safety Training, Animal Use Orientation (including Small Animal Use) at both Children's Hospital and Harvard Medical School.

During this time she also worked with the research technician in the laboratory of Dr. Alun Jones at Harvard Medical School, who maintains the multidrug-resistant MES-SA/Dx5 (uterine sarcoma) cell line that was used in previous studies in our laboratory, to learn the techniques required to establish and maintain drug resistant cell lines and the techniques required to measure uptake of the new radiotracers in these cells.

Because of space constraints at Harvard Medical School, it became necessary to establish a separate tissue culture facility in our laboratory. A suitable incubator was obtained on loan from Dr. Jones' laboratory, and much of the other required equipment was already available in our facility. Establishing this facility did, however, increase the time required to produce the target breast cancer cell lines.

Task 2. Establish and validate *in vitro* assay for multidrug resistant breast cancer, Months 3-12:

- a. Establish breast cancer cell lines
- b. Establish mdr breast cancer cell lines
- c. Validate parental and mdr breast cancer cell lines with  $^{99m}\text{Tc}$ -MIBI
- d. Validate parental and mdr breast cancer cell lines with prototype  $^{64}\text{Cu}$  PreH and cyclops complexes

This task is being pursued through two approaches; the rat MAT-B cell line and the human MCF-7 cell line. The MAT-B cell line has the advantage that is native to the Fischer rat – it was developed from a solid mammary adenocarcinoma - and is, therefore, expected to show more physiologically relevant perfusion when *in vitro* studies are carried out. The human-derived MCF-7 adenocarcinoma cell line is more relevant to the problem of human breast cancer, but *in vivo* studies must be carried out as xenografts in nude mice with the associated differences between the two species.

The first task was to establish wild-type and multidrug-resistant breast cancer cell lines. This task has been pursued with both the MAT-B and MCF-7 cell lines. The wild-type MAT-B cell line was obtained through our collaboration with Alun Jones, Ph.D., at Harvard Medical School. The mdr MAT-B sub-line was to have been developed in the fall and winter of 1999-2000 in Prof. Jones laboratory using standard procedures, but the associate in this laboratory who was to prepare these cells was on leave from approximately November, 1999, through March, 2000. One of her first objectives when she returned was to begin the process of preparing this resistant sub-line. This task is now expected to be completed this fall (2000). Both *in vitro* and *in vivo* studies will begin as soon as the cell line is validated.

Studies with the MCF-7 cell line were pursued in two ways. The first approach was to attempt to develop an mdr MCF-7 cell line in house from parental (non-resistant) MCF-7 cells obtained from ATCC, using the same approach used in Dr. Jones' laboratory on the MAT-B cell line. This approach is based on increasing the concentration of adriamycin in the culture media, starting at  $1 \times 10^{-9}\text{M}$  and then increasing the concentration to  $5 \times 10^{-9}\text{M}$ ,  $1 \times 10^{-8}\text{M}$ ,  $5 \times 10^{-8}\text{M}$ , etc. We found, however, that this rate of increase in the adriamycin concentration was too great. A significantly lower rate of increase, on the other hand, would require many months before an adequately resistant cell line was obtained. This approach was, therefore, set aside when the cells were at the  $1 \times 10^{-8}\text{M}$  level.

Alternatively, MCF-7 mdr cell lines were obtained from Drs. Grace Yeh (NIH) and Robert Gillies at the University of Arizona. In neither case, however, were our first efforts to cultivate these cells successful. In our hands, the Yeh cell lines did not grow in the presence of adriamycin. Based on correspondence with Dr. Yeh, we are developing a new working stock of these cells in the absence of adriamycin. Similarly, the first attempt to grow the resistant MCF-7 cells supplied by the Arizona Cancer Center was unsuccessful, apparently because of a problem with the cell line that was supplied. A second set of samples was recently obtained, and these are now being cultivated in our laboratory.

Because of the problems related to obtaining the resistant MCF-7 cell lines from other investigators, we are also now resuming our effort to develop resistant sub-lines in house. This effort was recently undertaken and will begin with the cells from the highest doxorubicin concentration achieved previously,  $1 \times 10^{-8}\text{M}$ . In this case the doxorubicin concentration will only be increased by a factor of two every 2-3 doubling times (4-6 days) with sufficient time allowed between increases for the cells to recover. This procedure is based on that used in Dr. Jones laboratory for the MAT-B cell line and is significantly slower than was used when we

initially tried to prepare the resistant sub-lines in house and is consistent with the rate of increase that is being used successfully in the MAT-B cells.

During the period when we have been addressing the problems associated with developing the drug resistant breast cancer cell lines, we have validated the *in vitro* assay techniques using the (previously established) MES-SA (parental) and MES-SA/Dx5 (resistant) uterine sarcoma cell lines. An abstract describing these results was submitted for presentation at the PacifiChem 2000 meeting. A copy of the abstract is included in the Appendix.

**Task 3.** Establish and validate *in vivo* assay for multidrug resistant breast cancer, Months 3-12:

- a. Establish breast cancer cell lines in animal model
- b. Establish mdr breast cancer cell lines in animal model
- c. Validate parental and mdr breast cancer cell lines with  $^{99m}\text{Tc}$ -MIBI
- d. Validate parental and mdr breast cancer cell lines with prototype  $^{64}\text{Cu}$  PreH and cycloplex complexes

*In vivo* testing of the new imaging agents will not be undertaken using a breast cancer model until the multidrug-resistant breast cancer cell lines are well established. Our revised estimate for this is Fall, 2000. Part of this delay is due to the problems that we have encountered in establishing the two mdr breast cancer cell lines and part is also due to the requirement that all new cell lines be extensively tested for rodent pathogens before they are used in animals that will be housed in the Children's Hospital animal facility. The cell lines can not be tested until they are well-established, and the *in vivo* studies can not be undertaken until the cell lines are tested.

## KEY RESEARCH ACCOMPLISHMENTS

- Recruited and trained research technician
- Established tissue culture facility in Children's Hospital Nuclear Medicine Laboratory
- Established human and murine breast cancer cell lines
- Multidrug resistant human and murine breast cancer cell lines are in process
- Validated techniques for *in vitro* measurement of uptake of new radiopharmaceuticals

## REPORTABLE OUTCOMES

### Manuscripts, abstracts, and presentations

"Synthesis and Characterization of Carrier-Free  $^{64}\text{Cu}$  Diiminedioxime Complexes - Potential PET Radiopharmaceuticals for Evaluating Multidrug Resistance." Packard AB, Kiani S, Guice E. Abstract accepted for oral presentation at PacifiChem 2000, December 14 - 19, 2000, Honolulu, Hawaii, in "Advances in Radiopharmaceutical Chemistry" symposium.

A copy of the abstract is appended to this report

### Cell lines

The MES-SA and MES-SA/Dx5 (human uterine sarcoma) cell lines are established and being used for method validation.

The MAT-B (rat mammary adenocarcinoma) parental cell line is established and the resistant subline will be available in Fall, 2000.

The parental and resistant MCF-7 (human mammary adenocarcinoma) cell lines are being evaluated at this time.

## CONCLUSIONS

At this early stage of the project, it is not yet possible to draw any conclusions about the validity of using  $^{64}\text{Cu}$ -based PET radiopharmaceuticals for the quantitative functional evaluation of multidrug resistance in breast cancer. We have, however, after some initial problems, been able to establish the foundation for the *in vivo* and *in vitro* evaluation of these new imaging agents in the remaining years of this project. These problems also provided the impetus to expand the range of cell lines that are being used for this evaluation, which will provide a more complete picture of the biological properties of the compounds. During this time we have also acquired a wider variety of  $^{64}\text{Cu}$  complexes to test when the cell lines are available. This expanded chemical diversity will also contribute to the overall goal of obtaining as complete as possible of the interrelationships between the chemical and biological properties of  $^{64}\text{Cu}$ -based PET radiopharmaceuticals for evaluating multidrug resistance in breast cancer.

## REFERENCES

N/A

# **APPENDICES**

(Erica Guice CV, PacificChem 2000 Abstract)



Erica Angelique Guice

15 Copley  
Roxbury, MA  
02119  
617-355-7560

guice@a1.tch.harvard.edu

Education:

August 1995- May 1999

University of Michigan  
Ann Arbor, MI 48109  
BS, Cell & Molecular Biology

August 1991- June 1995

Cass Technical High School  
2421 Second Ave.  
Detroit, MI 48201  
Chemical Biological

Work Experience:

October 1999-Present

Children Hospital  
Department of Nuclear Medicine  
300 Longwood Ave.  
454 Enders  
Boston, MA 02115

May 1997- May 1999

University of Michigan Hospitals  
Department of Neurology  
1500 E. Medical Center Drive  
5214 Cancer Center and Geriatric Center  
Ann Arbor, 48109

Activities:

April 1998-Present

Alpha Chi Sigma Professional Chemical Sciences Fraternity  
Alpha Beta Chapter  
1319 Cambridge Street  
Ann Arbor, MI 48105

April 1997- Present

Gamma Delta Iota Sorority  
Omega Chapter  
5731 Edge Waters Landing Ct.  
Burke, VA 22015

August 1995-May 1997

University of Michigan Marching Band  
Revelli Hall  
Ann Arbor, MI 48109

Resources available upon request.

## **Synthesis and Characterization of Carrier-Free $^{64}\text{Cu}$ Diiminedioxime Complexes – Potential PET Radiopharmaceuticals for Evaluating Multidrug Resistance**

The aim of this work is to develop  $^{64}\text{Cu}$ -based PET radiopharmaceuticals for the functional evaluation of multidrug resistance in breast cancer and other malignancies. We previously identified the lipophilic cationic copper(II) complexes of the diiminedioxime ligands as promising candidates for this application using low specific-activity  $^{64}\text{Cu}$  (2 mCi/mg) and carried out preliminary biological studies that confirm the potential utility of these complexes for this application. We have now developed a rapid synthesis (<5 min.) for use with high specific-activity (HSA)  $^{64}\text{Cu}$  (>105 mCi/mg) that produces these complexes in 90% yield and 90% radiochemical purity and characterized the products by HPLC and ITLC using the "cold" complexes as standards. In vitro stability was tested by equilibration of the  $^{64}\text{Cu}$ -complex with 2.5% BSA/PBS using a Sephadex G-50 column, which revealed no evidence of plasma binding or transchelation of  $^{64}\text{Cu}$  to albumin. Additional in vivo and in vitro studies are currently underway.